Cysteine Microplate Assay Kit

Cat #: orb707333 (manual)

Detection and Quantification of Cysteine Content in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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INTRODUCTION

Cysteine (CYS) is a sulfhydryl-containing amino acid and an important structural and functional part of proteins. In animals, cysteine is synthesized from transsulfuration of homocysteine, which is itself derived from metabolism of the amino acid methionine. Cystathionine β-synthase catalyzes condensation of homocysteine with serine to form cystathionine, which is deaminated and hydrolyzed by Cystathionine β-lyase to form cysteine and α-ketobutyrate. Because of its nucleophilic nature, the thiol group of cysteine has numerous biological functions. The formation of disulfide linkages between the thiol groups of cysteine residues helps to stabilize the tertiary and quaternarystructure of proteins. Cysteine, homocysteine (HCY), and other aminothiols exist in plasma in reduced, oxidized, and protein-bound forms, interacting with each other through redox pathways. Cysteine is the limiting precursor of the major intracellular antioxidant glutathione. The individuals with lower cysteine levels are more prone to damage from reactive oxygen species, which are generally removed either by thiols or by glutathione-linked enzymes. An elevated level of total cysteine also predicts adverse outcomes such as cardiovascular diseases and metabolic syndromes.

Cysteine Microplate Assay Kit is a sensitive assay for determining Cysteine in various samples. Cysteine concentration is determined by sodium tungstate dihydrate. The reaction products can be measured at a colorimetric readout at 600 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	8 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Dye Reagent: add 10 ml Dye Reagent Diluent to dissolve before use, then put it in boiling water for 10 minutes.

Standard: add 1 ml Distilled water to dissolve before use, the concentration will be 2 mol/L.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 600 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 10, 000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10, 000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Add 0.9 ml Assay buffer into 0.1 ml liquid sample, centrifuged at 10, 000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Reaction Buffer	80 µl	80 µl	80 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, incubate at room temperature for 15 minutes, measured at 600 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

CALCULATION

1. According to the protein concentration of sample

$$\text{Cysteine (mmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Cysteine (mmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Cysteine (mmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Cysteine (mmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the standard concentration, 2 mol/L = 2 mmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

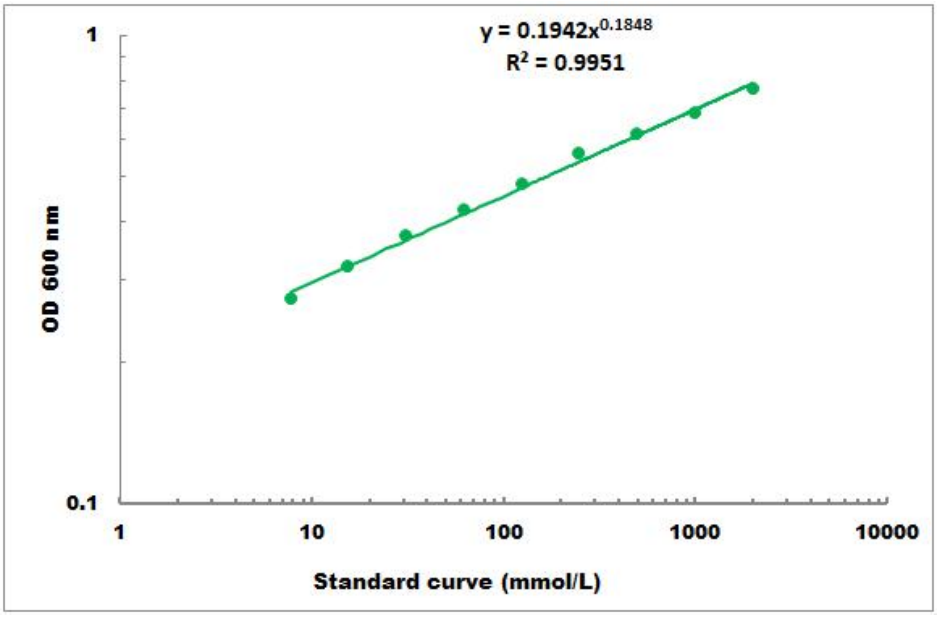
$V_{Standard}$: the volume of the standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 20 mmol/L - 2000 mmol/L

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